CYTOSKELETAL CHANGES IN ALZHEIMER'S DISEASE

Alzheimer’s disease is histopathologically characterized by extracellular amyloid deposits and abundant cytoskeletal abnormalities. By means of classical histochemical staining, especially silver staining, these abnormalities can be visualized in the perikaryon (the neurofibrillary tangle) and in neurites (dystrophic neurites). These latter phenomena may be associated with extracellular amyloid depositions, to form neuritic plaques, or they may lie outside the plaques, in which case they have been termed neuropil threads (1,2). The formation of these altered cytoskeletal constituents is a dynamic process, and silver staining offers but a restricted view of the final stages of these alterations. New vistas are offered by the use of antibodies directed against specific cytoskeletal structural proteins, e.g. microtubule-associated protein tau or ubiquitin (see below). At present, however, it seems difficult to fit all the old and new data into one mode of neuronal alterations in Alzheimer’s disease.

A limited number of immunoreactive neurons may also be found in the brains of nondemented individuals and of patients suffering from some other neurological diseases (3,4), which complicates the diagnostic specificity of such antibodies for Alzheimer’s disease. Moreover, the cytoskeletal alterations are distributed in a very heterogeneous way over cortical areas and layers, subcortical nuclei and different neuron populations. In the literature several hypothetical explanations for the different staining patterns have been given. It has been proposed that cytoskeletal staining preferentially takes place in cortical neurons or neurons projecting to the cortex (5). Diffuse labeling of the perikaryon of neurons lacking tangles has been considered to precede the formation of tangles (6,7). Cytoskeletal staining is thought to be a sign of impending neuronal death, not only in Alzheimer’s disease (7–9), but also in normal brain development (9,10). A causal relationship between the
occurrence of dystrophic neurites and deposition of amyloid has been presumed, but not confirmed (11).

THE HUMAN HYPOTHALAMUS

The human hypothalamus is a unique structure for testing the different hypotheses on the pathophysiological meaning of the cytoskeletal markers in Alzheimer’s disease. In the first place it contains both neurons that project to the cortex, e.g. the nucleus basalis of Meynert (NBM), and neurons that do not project to the cortex, e.g. the supraoptic nucleus (SON), the paraventricular nucleus (PVN), the suprachiasmatic nucleus (SCN) and probably the sexually dimorphic nucleus (SDN) and the nucleus tuberis lateralis (NTL), so that the hypothesis that mainly systems connected to the cortex are affected (5) can be tested in this structure. The human hypothalamus also offers the possibility of testing hypotheses on the relation between cytoskeletal changes and the fate of neurons in aging and dementia. The NBM is generally assumed to degenerate in aging and even more so in Alzheimer’s disease (see below). In the SCN, which is the clock of the hypothalamus, a decrease in cell number occurs in normal aging, which is even more pronounced in Alzheimer’s disease (12). The sexually dimorphic nucleus (SDN) shows a decrease in cell number which is similar in aging and in Alzheimer’s disease (13,14). On the other hand, no cell loss is found in the SON, PVN and NTL either in Alzheimer patients or in controls (15,16).

METHODS

Cytoskeletal alterations in aging and Alzheimer’s disease were related to cell counts in the different hypothalamic nuclei by using the following four antibodies:

60e, a polyclonal antibody raised against isolated neurofibrillary tangles which was also used following pretreatment of the sections with formic acid in order to uncover epitopes on tangles (17). This antiserum contains antibodies to normal tau and to an abnormal form of tau, but is not phosphatase-sensitive and does not react with ubiquitin (18).

tau-1, a monoclonal antibody to the microtubule-associated protein tau (19). Dephosphorylation of the tissue sections with alkaline phosphatase (86 μg/ml) was performed prior to immunocytochemistry, since it increases the number of tangles and plaques recognized by the antibody (20). Without dephosphorylation no staining was obtained with tau-1 in the hypothalamic nuclei of Alzheimer patients or controls.

3-39, a monoclonal antibody raised against isolated neurofibrillary tangles and directed against ubiquitin (7,21–23).

Alz-50, a monoclonal antibody which reacts with tau and A68 (24–27).

For the present study 6-μm paraffin sections of formalin-fixed hypothalami were used from 13 neuropathologically confirmed Alzheimer patients and 13 age-
and sex-matched controls. The age of the controls ranged from 47 to 91 years and that of the Alzheimer patients from 46 to 94 years.

RESULTS (Figures 1, 2)

The nucleus basalis of Meynert (NBM) is an origin of the cholinergic innervation of the neocortex. It is as yet unclear whether the degenerative changes in the NBM in Alzheimer’s disease contribute to the memory disturbance in this condition (28) and whether neuronal loss in the NBM occurs during normal aging (29,30). The number of large cholinergic neurons is generally reported to decrease in Alzheimer’s disease (29-31). However, since at the same time the proportion of small neurons is increasing (32,33), many of the large cells in the NBM may be shrunken instead of dead. NBM neurons of Alzheimer patients showed abundant staining, compared to those of controls, with antibodies 60e (even more so after pretreatment with formic acid), anti-tau-1 (after dephosphorylation) and 3-39. After dephosphorylation, tau-1 visualized dystrophic neurites as well as neuritic plaques. The most obvious difference between Alzheimer patients and controls was observed with Alz-50 staining. In controls, NBM neurons are generally unstained, except for some neurons in a 63-year-old man, and some neurons and dystrophic neurites in a 90-year-old woman. In Alzheimer patients the NBM was generally full of diffusely staining perikarya, tangles and dystrophic neurites. Therefore, the NBM is a very suitable brain structure for an immunocytochemical validation of the neuropathological diagnosis of Alzheimer’s disease. The NBM changes are compatible with the various pathophysiological hypotheses mentioned before.

The supraoptic nucleus (SON) can be regarded in many respects as the opposite of NBM. The neurosecretory neurons of the SON produce the neuropeptides vasopressin and oxytocin, which are released into the circulation in the neurohypophysis. They form a population of extremely stable cells. Neither in the course of normal aging nor in Alzheimer’s disease was any significant loss in neurons or total cell number observed (15). None of the four antibodies mentioned earlier revealed any cytoskeletal staining in the SON. The differences between the cytoskeletal alterations observed in the NBM, which is affected in Alzheimer’s disease, and those observed in the SON, which is spared in this condition, are in agreement with the various hypotheses on the possible meaning of cytoskeletal staining mentioned in the introduction. However, results obtained from other hypothalamic nuclei (see below) are inconsistent with most of these hypotheses.

The paraventricular nucleus (PVN) contains neurosecretory neurons that project to the neurohypophysis and median eminence. In this nucleus no loss of total cell number was observed either in aging or in Alzheimer’s disease (15). Yet, both in controls and in Alzheimer patients diffuse cytoplasmic staining was regularly observed with 60e following formic acid pretreatment, with tau-1 following dephosphorylation, with 3-39 and, most clearly, with Alz-50. This staining was also present in young controls (Figure 1F).
Figure 1. (A–D) Nucleus basalis of Meynert of a 45-year-old male Alzheimer patient (no. 86.3645.2) stained with (A) Alz-50—note plaques, tangles and dystrophic neurites; (B) 60e, directed against neurofibrillary tangles, following pretreatment with formic acid; (C) 3-39, raised against tangles and directed against ubiquitin; (D) tau-1 following dephosphorylation; note plaques, tangles and dystrophic neurites. (E) Nucleus tuberalis lateralis, stained with Alz-50. Note tangles and dystrophic neurites (female Alzheimer patient no. 88.325, 64 years of age). (F) Paraventricular nucleus of a 47-year-old male control (no. 87.271). Note the cytoplasmatic staining of the neurons. Scale bar 200 μm; III, third ventricle
Figure 2. (A,B) Suprachiasmatic nucleus of 64-year-old female Alzheimer patient (no. 87.494.2) stained with anti-vasopressin (A; note the few positive cells in the degenerated nucleus indicated by arrows) and with the anti-ubiquitin 3-39 (B); III, third ventricle. (C,D) Sexually dimorphic nucleus of 70-year-old female Alzheimer patient no. 8560 stained with tau-1 following dephosphorylation (C) and with 60e following formic acid pretreatment (D); note the cell body staining (C,D) and dystrophic neurites (D). Scale bar 200 μm
Therefore, in PVN diffuse perikaryal labeling of neurons may not be indicative of intraneuronal abnormalities preceding the formation of tangles and impending cell death. Interestingly enough, Alz-50 positive neuropil threads were observed only in the PVN of Alzheimer patients and not in that of controls, except for the presence of some neuropil threads in one 90-year-old female control.

The lateral tuberal nucleus (NTL) is supposed to be present only in humans and higher primates. In adulthood it contains 60,000 neurons, whereas in Huntington’s disease this number may be reduced to less than 10,000 (34). Pathological changes in the NTL have also been described in dementia with argyrophilic grains throughout the neuropil and silver-staining coiled bodies, containing straight filaments (35). In both Alzheimer’s disease and Huntington’s disease, dementia is associated with severe loss of weight despite normal or even increased food intake. In the condition described by Braak and Braak (35), the severe NTL pathology is also accompanied by cachexia (H. Braak, personal communication). Therefore, the NTL may play a role in feeding behavior and metabolism.

In Alzheimer’s disease, no alteration in the number of NTL neurons was found. Neurofibrillary tangles were rare in conventional silver stains. Yet, immunocytochemical staining by Alz-50 showed such an abundant reactivity of both perikaryal and dystrophic neurites that the NTL of Alzheimer patients could even be recognized with the naked eye (34). It seems, therefore, that the NTL represents a brain area in which Alzheimer’s disease affects the neurons in a limited way, without progression toward the classical silver staining tangles or neuronal loss. The observations in this nucleus show that even a very strong Alz-50 staining does not predict impending neuronal death, since in Alzheimer’s disease neuron counts in the NTL did not show any decline in numbers. The other three antibodies have not been applied to the NTL.

The sexually dimorphic nucleus (SDN) of the preoptic area was first described by Gorski et al (36) in the rat. In the rat the SDN is three to eight times larger in the male than in the female, due to differences in perinatal steroid levels (37). The SDN is involved in aspects of male sexual behavior (e.g. mounting), not only in male, but also in female rats (38, 39). In the human brain the SDN is twice as large in males as in females and contains twice as many cells (40). The SDN is identical to the ‘intermediate nucleus’ described by Braak and Braak (41). Cell numbers in the male SDN decrease sharply after 50 years of age, while in females a phase of marked cell loss sets in approximately around the age of 70 (13, 14). The decrease in cell numbers in this nucleus later in life might be related to the hormonal changes which accompany both male and female senescence (14), and to the decrease in male sexual activity. Yet it is not clear whether the hormonal changes would be the cause or effect of the observed cell loss in this nucleus. Cell numbers in the SDN of Alzheimer’s patients were found to be within the normal range for age and sex so that this condition does not seem to affect the SDN in any specific way (13).

Some positive cell bodies were found in the SDN of a small number of Alzheimer patients with 60e, tau-1 and 3-39 staining. Alz-50 staining revealed some positive SDN neurons and dystrophic neurites in Alzheimer patients, whereas controls were
negative for all four antibodies (except for a few Alz-50 positive neurons in one control patient, a 63-year-old male). Since the SDN cell numbers decrease in a similar way in Alzheimer patients and controls, cytoskeletal staining cannot be related in a simple way to cell death in the SDN.

The suprachiasmatic nucleus (SCN) is considered to be the major circadian pacemaker of the mammalian brain, coordinating hormonal and behavioral circadian rhythms (42). Age-related changes in circadian rhythms have been reported in humans as well as in animals (43). Among the most prominent changes is a fragmentation of sleep-wakefulness patterns in senescence, a phenomenon that is even more pronounced in Alzheimer's disease (44). Sleep disturbances often lead to hospital admission of the elderly (45). Total SCN cell numbers and numbers of vasopressin-expressing neurons were, therefore, determined during aging and in Alzheimer's disease. A marked decrease was found in total SCN cell numbers and in the number of vasopressin neurons in subjects of 80-100 years of age, while in Alzheimer's disease these changes were still more dramatic (46,47).

In spite of the pronounced SCN changes in Alzheimer's disease, the cytoskeletal alterations were generally only minimal, with the exception of one 64-year-old female Alzheimer patient, whose entire SCN was stained with tau-1 and 3-39 but not with 60e or Alz-50. Several Alzheimer patients had few positive SCN cells with 60e following formic acid treatment, with tau-1 after dephosphorylation, and with 3-39, but the stainings were far from impressive. Alz-50 showed some positive perikarya, whereas dystrophic neurites were hardly seen at all.

CONCLUSIONS

Some diffuse cytoplasmatic staining was also observed in young nondemented controls, raising doubts about the specificity of the antibodies for Alzheimer’s disease. This phenomenon was most conspicuous in PVN neurons with Alz-50. However, Alz-50 staining of dystrophic neurites was clearly observable only in Alzheimer patients, so staining of dystrophic neurites seems to be more specific for the histopathological diagnosis of Alzheimer’s disease than perikaryal staining.

Perikaryal staining is not necessarily indicative of neuronal abnormalities preceding the formation of tangles or impending cell death, as is clear from the PVN staining in young controls and the NTL staining in Alzheimer patients. Bondareff et al (48), using thioflavin-S staining, also came to the conclusion that the appearance of tangles does not necessarily herald the demise of a neuron in Alzheimer’s disease.

The very strong Alz-50 staining in the NTL, from which no direct cortical connections are known, raises doubts about the idea that cytoskeletal changes would be restricted to cortical areas or nuclei projecting to the cortex. The same can be said about the SCN and SDN staining observed in some Alzheimer patients, since these nuclei are not considered to be directly connected to the cortex either.

Consequently, the pathophysiological meaning of cytoskeletal staining in Alzheimer’s disease is at present far from clear.
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REFERENCES


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